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Clinical, Microbiological, and Histological Manifestations of *Streptobacillus moniliformis*-Induced Arthritis in Mice

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Intravenous inoculation of *Streptobacillus moniliformis* into mice resulted in an infection in which the predominant feature was progressive polyarthritis that rendered some joints immobile within 6 months. No migration of arthritis from joint to joint or remission and exacerbation were apparent. Viable organisms were apparently removed by the host from blood, liver, and spleen within 28 days post-inoculation but persisted in joints for approximately 6 months in some animals. Specific antibody was detectable by complement fixation 7 days post-inoculation and persisted throughout the course of the disease. The inflammatory response, which was initiated by the appearance of neutrophils in the joint space within 24 h of inoculation, culminated in obliteration of the joint space by fibrosis and exostosis.

Streptobacillus moniliformis is a nutritionally fastidious, gram-negative bacterium that is potentially pathogenic for humans and for some laboratory animals. The disease produced by this organism in humans has been known as Haverhill fever (2) and rat bite fever (2, 4, 8, 11) because its primary mode of transmission seems to be via rat bite. One of the predominant features of *S. moniliformis* infection in humans has been the development of chronic arthritis which persists for years, with periods of remission and exacerbation (2). Reported complications of the disease in humans have included subacute bacterial endocarditis (2, 8, 12, 14) and brain abscesses (13). In animals, particularly mice, the infection has also been characterized by the development of arthritis (1-3, 5-7, 9, 10). Because most studies have been terminated within 1 month after infection (1-3, 5-7, 9, 10, 15), the duration of the arthritis in mice is unknown despite one report which indicated the persistence of the organism in a mouse for 16 months (11). At the present time, the mechanisms by which *S. moniliformis* produces disease, and specifically arthritis, are unknown. In this paper, we describe some of the clinical, microbiological, and histological manifestations of this infection through its acute phase and to its resolution and evaluate the murine system as a potential model for the study of infectious arthritis.

MATERIALS AND METHODS

Microorganism. An *S. moniliformis* strain isolated from the middle ear of an albino rat was used. The organism was maintained by growth in tryptose phosphate broth (Difco Laboratories, Detroit, Mich.) supplemented with 20% horse serum (Difco or Microbiological Associates, Bethesda, Md.) and 10% yeast extract (Difco). An inoculum was prepared by diluting a 24-h broth culture in fresh broth to give the desired number of organisms and administered intravenously in the lateral tail vein. Unless otherwise indicated, approximately 10^8 organisms were injected. The necessary amount of dilution was predetermined by plate counts, direct microscopic counts, and spectrophotometric measurements. Stock cultures were preserved by freezing in 20% glycerol and by lyophilization.

Animals. Male or female noninbred, Swiss-Webster mice (Harlan Industries, Indianapolis, Ind.) were grouped by weight (25 to 30 g). Animals were fed Purina Lab Chow and water ad libitum.

Clinical course of infection. Six groups of 10 mice each were inoculated with different numbers of organisms to determine the clinical course of the infection. Each animal was examined for development of grossly apparent (red and swollen) arthritic joints. Swelling in each arthritic joint was determined by subtracting vernier dial caliper measurements of normal joints from contralateral grossly arthritic joints. Rates of mortality and arthritic development and distribution and persistence of lesions were determined for each dosage level.

Clearance and persistence of organisms. Groups of 10 infected mice each were serially examined for the presence of organisms in the blood, livers, spleens, and joints. Blood was obtained by cardiac puncture, diluted in fresh media, and plated on supplemented tryptose phosphate agar. Spleens, livers, and joints were dissected, weighed, homogenized in Ten Broeck homogenizers, diluted, and plated. Joints were minced with scissors before being homogenized.

No attempt was made to quantify the number of organisms per joint. Initially, at 4 h post-inoculation, all joints appeared normal; therefore, both knee and elbow joints from each mouse were cultured. After 48 h, joints were classified either as grossly arthritic or normal.

Histopathology. Joints for histopathological examination were arbitrarily selected from inoculated animals before the development of lesions; thereafter, joints with gross lesions were selected. The joints were fixed in 10% phosphate-buffered Formalin, decalcified in HCl, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Serology. Normal and infected mouse serum antibody titers were determined by complement fixation with the Cooke microtiter system (Cooke Engineering Co. [Dynatech Corp.], Alexandria, Va.). Antigens were prepared by sonication with a sonic dismembrator (Artec Systems Corp.) of whole cells previously washed five times in phosphate-buffered saline (pH 7.2).

RESULTS

Clinical course of infection. The responses of noninbred, Swiss-Webster mice to *S. moniliformis* administered intravenously appeared to be dose dependent. Of 10 animals inoculated with 5×10^9 organisms, 8 died within 14 days, whereas only 1 of 10 animals inoculated with 5×10^7 organisms died (Table 1). Similarly, animals developed clinical arthritis more frequently and more rapidly when inoculated with large doses (Table 2). All 10 animals receiving 5×10^9 organisms developed extensive arthritis, with an average of five joints affected per animal. Development of arthritis in this group was complete by 4 days post-inoculation. Of 10 animals inoculated with 5×10^8 organisms, 8 developed arthritis, with an average of three joints affected per mouse, whereas 3 of 10 animals inoculated with 5×10^7 organisms developed arthritis, with an average of 1.3 joints affected per mouse. Animals in both of these groups exhibited a maximum number of arthritic joints at 7 days. Grossly arthritic joints persisted in the animals for 161 days, with some reduction in actual average number per animal. The joints at 161 days were not red, and the enlarged appearance may have reflected chronic arthritis. The clinical course of the arthritic lesions was characterized by an increase in the size of the affected joint until it reached a maximum size within 21 days after the initial inoculation, followed by slow and incomplete healing of the arthritic joints. Approximately 80% of the arthritic joints initially evident were still evident at 161 days post-inoculation. New sites of arthritis did not develop after the initial acute phase of the disease, nor did any of the existing arthritic joints exhibit any obvious regression followed by exacerbation.

TABLE 1. *Dose-dependent effect of S. moniliformis on mortality of laboratory mice*

Dose (no. of organisms)	Cumulative no. dead/no. injected at indicated days post-inoculation				
	2	4	7	14	21
5×10^9	0	0	2/10	8/10	8/10
5×10^8	0	0	0	2/10	2/10
5×10^7	0	0	0	1/10	1/10

TABLE 2. *Development and persistence of S. moniliformis-induced arthritis in mice*

Dose[a]	No. of arthritic animals/no. inoculated	Last day post-inoculation that new arthritic joints appeared	Avg no. of lesions per afflicted animal[b]	Avg no. of arthritic joints per surviving animal[c]
5×10^9	10/10	4	5.0	4.0
5×10^8	8/10	7	3.0	2.4
5×10^7	3/10	7	1.3	0.7

[a] Number of organisms in 0.1 ml of inoculum administered to each animal.

[b] At height of arthritis. Represents those joints exhibiting swelling and perhaps permanent deformity. Joints did not exhibit erythema.

[c] At 161 days post-inoculation.

Clearance and persistence of organisms. The number of organisms in the blood, livers, and spleens declined continuously from the time of inoculation until about 28 days, at which time clearance of organisms was virtually complete (Fig. 1). Similarly, virtually 100% of tissue samples from animals contained organisms until day 14, with a gradual decline most evident at 28 days. Tissues from 15 animals subsequently sampled at 35, 42, and 56 days (5 at each time) were culture negative.

Organisms were isolated from joints at 4 h post-inoculation and from some grossly arthritic joints approximately 6 months afterward (Table 3). Virtually all cultures from grossly arthritic joints were positive from 2 to 56 days after infection, with a slight reduction to 10 of 14 positive at 100 days and a greater reduction to 3 of 14 positive at 161 days. Subsequent attempts to isolate *S. moniliformis* from grossly arthritic joints were unsuccessful.

Antibody development. Circulating antibodies were first detected 7 days post-inoculation and persisted at a comparatively uniform level between 14 and 200 days (Table 4).

Histopathological findings. Fibrinopurulent exudate appeared within joint spaces and adjacent periostea of affected mice within 24 h post-inoculation. The intensity of this host response increased significantly within the subse-

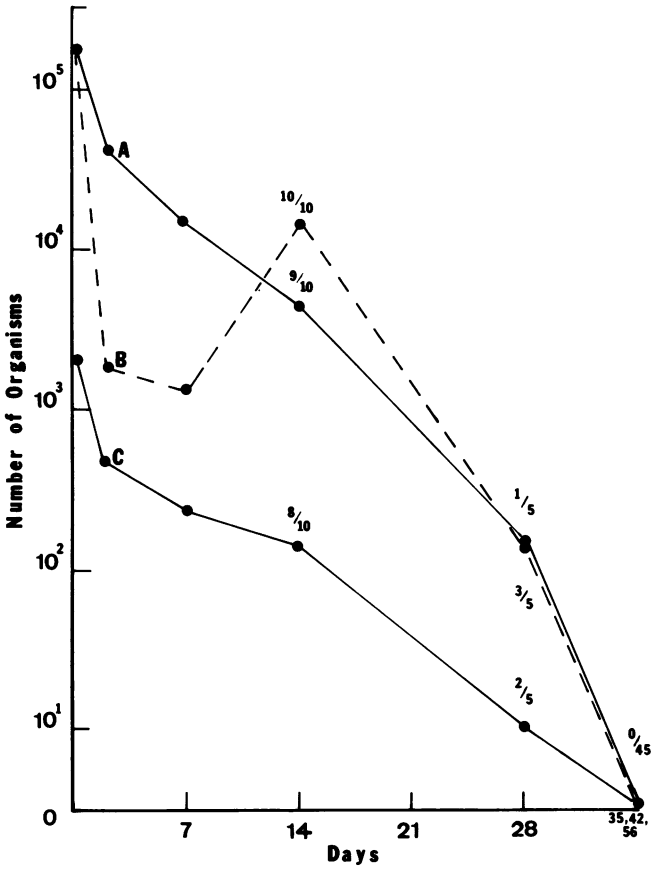

FIG. 1. *Clearance of S. moniliformis from the spleen (A), liver (B), and blood (C). Numbers of points represent the numbers of animals culture positive relative to the numbers of animals examined. The numbers of organisms in the spleen, liver, and blood are the numbers per spleen, per gram of liver, and per milliliter of blood, respectively.*

TABLE 3. *Isolation of S. moniliformis from joints of mice*[a]

Time post-inoculation[b]	No. of animals tested	No. of joints culture positive/no. of joints examined	
		Normal joints	Arthritic joints
4 h	10	37/38	ND[c]
2	10	13/24	10/12
7	10	4/15	20/21
14	10	4/13	23/23
28	10	0/10	10/10
56	12	0/12	14/15
100	10	ND	10/14
161	14	ND	3/14
200	10	ND	0/10

[a] Animals were inoculated with 2.4×10^8 organisms.
[b] In days, unless otherwise indicated.
[c] ND, Not determined.

TABLE 4. *Titers of complement-fixing antibodies in mice experimentally infected with S. moniliformis*

Days post-inoculation	No. of animals tested	Mean antibody titer ($\log_{10}$)
4	10	0
7	10	0.69
14	10	1.89
28	10	1.98
56	10	2.12
100	8	1.75
161	5	2.04
200	10	1.74

quent 24-h period and remained the predominant cellular reaction until the appearance of numerous macrophages on day 4. Periarticular abscesses and necrosis of adjacent skeletal muscle were observed on day 7. Within 2 weeks, a significant degree of periostitis and new bone

formation was evident; this was accompanied by an admixture of neutrophils, lymphocytes, and macrophages (Fig. 2). By week 3, fibrous connective tissue proliferation began along with sustained periosteal bone formation. In some areas, newly formed bone and dense fibrous connective tissue obliterated the joint space. Although diminishing gradually, scattered foci of neutrophils and macrophages remained persistent components of the inflammatory response throughout the entire study. At no time was villous hyperplasia or surface erosion of joints noted as a component of this reactive process.

DISCUSSION

Intravenous inoculation of *S. moniliformis* into noninbred, Swiss-Webster mice resulted in a disease characterized by polyarthritis which was fatal in heavily infected animals. The degree of polyarthritis, based on the average number of arthritic joints per infected mouse, was dose dependent. Those animals receiving the heaviest inocula developed arthritis more rapidly and extensively, died more rapidly, and generally developed chronic ankylosing arthritis, with permanent immobility of the affected joints.

Development of arthritis in infected mice seemed to correlate with localization and persistence of organisms in the joints. The organisms persisted in the blood, liver, and spleen long enough to allow adequate distribution to the joints. Although development of circulating antibodies apparently aided in the subsequent clearance of organisms from the blood, liver, and spleen, organisms persisted in joints for approximately 3 months after the development of a comparatively high titer of antibodies. As the organisms were eliminated from the joints, apparent arthritis persisted in the form of an immobilized joint structure.

The joint lesions occurring in *S. moniliformis*-infected mice are similar to those reported in rats (10) except that the lesions in mice seem to arise within the joint space and adjacent periosteum rather than within the epiphyses of the long bones and adjacent periosteum. The lesions appear to be consistent with those produced by many other infectious agents affecting the joints via the vascular system. The initial fibrinopurulent exudate and subsequent lymphocytic and histiocytic infiltration, with scattered focal infiltrates persisting throughout the study period, the isolation of organisms from these long-term lesions, and the absence of villous hyperplasia and surface erosion are characteristic of arthritis caused by chronic infection. The significance of this infection relative to others producing similar arthritis is the persistence of the organisms in the joints.

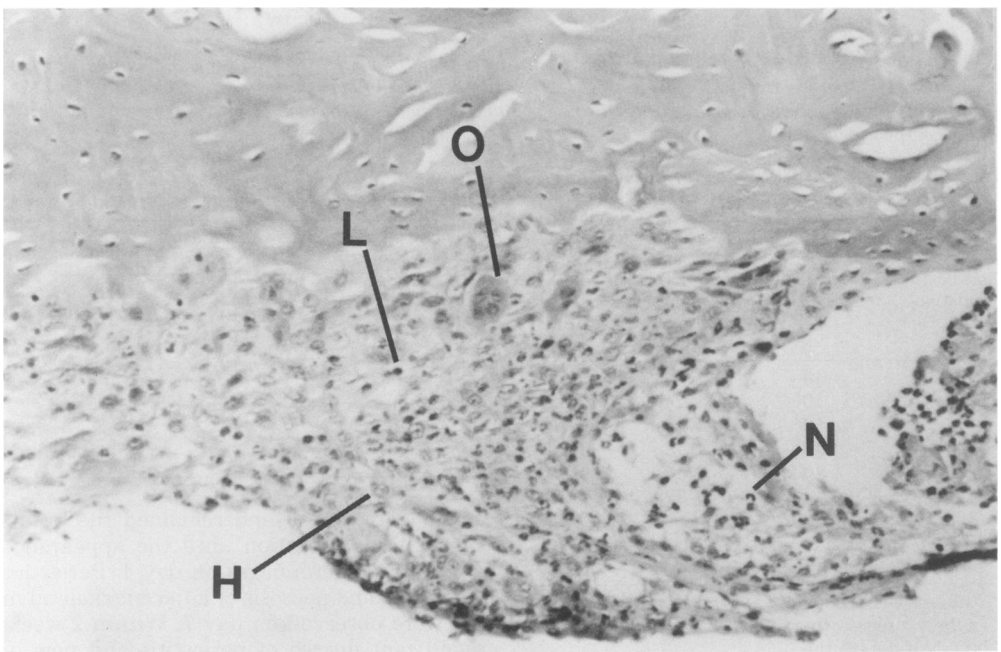

FIG. 2. *Osteoclasts (O), neutrophils (N), lymphocytes (L), and histiocytes (H) on the articular surface of an affected joint of a mouse infected 2 weeks previously. Hematoxylin and eosin stain. ×240.*

S. moniliformis-induced arthritis in noninbred, Swiss-Webster mice does not represent a model characteristic of human rheumatoid arthritis. Rather, it seems to be more characteristic of other bacterially induced arthritic conditions caused by blood-borne organisms. *S. moniliformis* may be unique, however, in that the primary manifestation of infection in mice is arthritis. Consequently, questions relative to the mechanism whereby the organism localizes in the joint tissues and persists in those joints even in the presence of circulating antibodies need to be answered.

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